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b) transfecting/transducing *in vitro* a population of chondrocyte cells with said recombinant vector, resulting in a population of transfected/transduced connective tissue cells; and

c) injecting a composition comprising the transfected/transduced population of chondrocyte cells and a pharmaceutically acceptable carrier without scaffolding into a joint space of a mammal such that expression of the DNA sequence encoding TGFβ1 or BMP within the joint space occurs resulting in the generation of hyaline cartilage in the joint space.

14. (Amended) The method of claim 13, wherein said transfection is accomplished by liposome encapsulation, calcium phosphate coprecipitation, electroporation and DEAE-dextran mediation.

REMARKS

Claims 1-5 and 13-15 are pending in the application. The amendments to the specification are made to reinsert the term "mesenchyme" into the application. The amendments to the claims serve to further clarify the present invention. Support for the insertion of "without scaffolding" in claims 1 and 13 can be found at *inter alia*, page 12, para no. 83. No new matter has been inserted into the application. Accordingly, entry of the amendments to the application is respectfully requested.

Specification

The Examiner has objected to the specification for containing new matter. The presently submitted amendments to the specification effectively cancels what the Examiner has deemed as new matter. Accordingly, this objection has been overcome.

Claim Objections

The Examiner has objected to variously the margin length of the page containing claims 9 and 11. Claims 9 and 11 have been canceled. Accordingly, this objection has been overcome.

Rejection Under 35 U.S.C. 112, first paragraph

Claims 1-22 have been rejected under 35 U.S.C. 112, first paragraph because the specification allegedly does not provide enabling disclosure for treating arthritis, using any protein of the TGF superfamily, any connective tissue cells or regenerating any connective tissue. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The presently claimed invention is directed to a method of regenerating hyaline cartilage, comprising:

a) generating a recombinant viral or plasmid vector comprising a DNA sequence encoding transforming growth factor β1 (TGF-β1) or BMP operatively linked to a promoter;

b) transfecting/transducing *in vitro* a population of chondrocyte cells with said recombinant vector, resulting in a population of transfected/transduced connective tissue cells; and

c) injecting a composition comprising the transfected/transduced population of chondrocyte cells and a pharmaceutically acceptable carrier without scaffolding into a joint space of a mammal such that expression of the DNA sequence encoding TGFβ1 or BMP within the joint space occurs resulting in the generation of hyaline cartilage in the joint space.

The present application is also directed to a method of treating osteoarthritis comprising:

a) generating a recombinant viral or plasmid vector comprising a DNA sequence encoding transforming growth factor β1 or BMP operatively linked to a promoter;

b) transfecting *in vitro* a population of chondrocyte cells with said recombinant vector, resulting in a population of transfected/transduced chondrocyte cells; and

c) transplanting said transfected/transduced chondrocyte cells without scaffolding by intraarticular injection to an osteoarthritic joint space of a mammalian host, such that expression of said DNA sequence within said joint space results in regenerating connective tissue.

In the presently claimed invention, chondrocytes are transfected or transduced with TGFβ1 or BMP and the resultant composition along with a pharmaceutical carrier which does not include scaffolding or any other such physical matrix is injected into the joint space to regenerate hyaline cartilage. Using chondrocytes is disclosed at least at pages 5 and 9 in the present application. Use of BMP protein is discussed at least at page 5 in the present specification.

Applicants respectfully submit that given the guidance presented in the instant specification, a person of ordinary skill in the art would know how to transfect or transduce chondrocyte with genes encoding TGFβ1 or BMP and inject them into the knee joint. These are discussed in detail in which fibroblast/TGFβ1 has been specifically and without limitation exemplified. However, the application explicitly allows for the use of chondrocytes to regenerate cartilage following the same or similar method as using fibroblasts applying well-known molecular biological techniques. Thus, applicants submit that the present application provides a fully enabling description of the presently claimed invention.

Applicants respectfully submit that given the guidance presented in the instant specification, the application provides an enabling disclosure for treating osteoarthritis especially considering the regeneration of hyaline cartilage in rabbits as exemplified in the

present application. Regeneration of hyaline cartilage can be thought of as *de facto* treating osteoarthritis because osteoarthritis is caused by the mechanical wearing away of the cartilage, and thus regeneration of cartilage replenishes the degraded cartilage to treat osteoarthritis. Furthermore, no immuno-rejection is seen by the inventive procedure. Thus, applicants submit that the present application provides a fully enabling description of the presently claimed invention. Withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. 112, second paragraph

Claim 8 has been rejected as being indefinite. Claim 8 has been canceled. Thus, this rejection has been overcome.

Rejection Under 35 U.S.C. 102(b) Over Agrawal (Agrawal et al., 1995, Indian J.Exp.Biol. Vol.33, pages 708-709)

Claims 16-21 have been rejected under 35 U.S.C. 102(b) as being anticipated by Agrawal. Claims 16-21 have been canceled. Thus, this rejection has been overcome.

Rejection Under 35 U.S.C. 103(a) Over Agrawal alone

Claims 16-22 have been rejected under 35 U.S.C. 103(a) as being obvious over Agrawal. Claims 16-21 have been canceled. Thus, this rejection has been overcome.

Rejection Under 35 U.S.C. 103(a) Over Naughton '477 in view of Ikeda (Ikeda et al., Sept. 1998, J. Rheumatol., Vol. 25, pages 1666-1673) and van Beuningen (van Beuningen et al., Sept. 1998, Osteoarthritis and Cartilage, Vol. 6, pages 306-317)

Claims 1-22 have been rejected under 355 U.S.C. 103(a) as being obvious over Naughton '477 in view of Ikeda and van Beuningen. Applicants traverse this rejection. Reconsideration and withdrawal of this rejection is respectfully requested.

Naughton '477

Naughton '477 discloses placing an artificial framework such as a scaffold together with periosteal/perichondral tissue and stromal cells near the cartilage defect region such that stromal cells are partly trapped in the scaffold. However, Naughton '477 fails to disclose or suggest injecting a cell composition into the joint without the use of an artificial framework.

Ikeda

Ikeda discloses intra-articularly injecting TGFβ1 containing adenovirus in an *in vivo* gene therapy methodology. However, Ikeda fails to disclose or suggest a cell-mediated gene delivery system as in the presently claimed invention.

van Beuningen

van Beuningen discloses increased proteoglycan gynthesis by injecting the protein form of TGFβ1. However, van Beuningen fails to disclose or suggest a cell-mediated gene delivery system.

The Examiner has failed to establish a *prima facie* case of obviousness of the presently claimed invention over the cited references. Naughton '477 requires using a physical object such as a scaffold for its transplantation method. In contrast, the presently claimed method is an injection method, and therefore does require the use of any such physical objects. Neither Ikeda nor van Beuningen cure this deficiency. Therefore, the presently claimed invention is not obvious over the cited references.

Double Patenting Under 35 U.S.C. 101

Claims 1-22 have been provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1-22 in co-pending Application 09/702,718. Applicants respectfully request the Examiner to hold this rejection in abeyance until one of these applications is in condition for allowance.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 07-1853 during the pendency of prosecution of this application. Should such additional fees be associated with an extension of time, applicant respectfully requests that this paper be considered a petition therefor. A duplicate of this paper is enclosed for the Deposit Account, should it be needed.

Respectfully submitted,

SQUIRE, SANDERS & DEMPSEY L.L.P.

Dated: July 24, 2002

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VERSION MARKED TO SHOW CHANGES MADE

In the Specification:

At page 4, beginning at line 27, please amend the following paragraph as follows:

The connective tissue cells include, but are not limited to, fibroblast cells, mesenchymal cells, osteoblasts, or chondrocytes. The fibroblast cells may be NIH 3T3 cells or human foreskin fibroblast cells.

At page 5, beginning at line 22, please amend the following paragraph as follows:

The present invention is also directed to a connective tissue cell line comprising a recombinant viral or plasmid vector comprising a DNA sequence encoding a member of the transforming growth factor superfamily. The connective tissue cell line may include, but is not limited to, a fibroblast cell line, a mesenchymal cell line, a chondrocyte cell line, an osteoblast cell line, or an osteocyte cell line. The fibroblast cell line may be a human foreskin fibroblast cell line or NIH 3T3 cell line.

At page 8, beginning at line 22 please amend the following paragraph as follows:

As used herein, the term "connective tissue cell" or "cell of a connective tissue" include cells that are found in the connective tissue, such as fibroblasts, cartilage cells (chondrocytes), and bone cells (osteoblasts/ osteocytes), which secrete collagenous extracellular matrix, as well as fat cells (adipocytes) and smooth muscle cells. Preferably, the connective tissue cells are fibroblasts, cartilage cells, and bone cells. More preferably, the connective tissue cells are fibroblast cells. Connective tissue cells also include mesenchymal cells which are also known as immature fibroblasts. It will be recognized that the invention can be practiced with a mixed culture of connective tissue cells, as well as cells of a single

type. It is also recognized that the tissue cells may be treated such as by chemical or radiation so that the cells stably express the gene of interest, preferably TGF-β.

In the Claims

Please cancel claims 6-12, and 16-22 without prejudice or disclaimer of the subject matter thereof.

Please replace 1-5 and 13-14 with the following amended claims 1-5 and 13-14:

1. (Amended) A method of treating [arthritis] osteoarthritis comprising:

a) generating a recombinant viral or plasmid vector comprising a DNA sequence encoding [a member of a] transforming growth factor β1 or BMP [superfamily of proteins] operatively linked to a promoter;

b) transfecting *in vitro* a population of [cultured connective tissue] chondrocyte cells with said recombinant vector, resulting in a population of [transfected] transfected/transduced [connective tissue] chondrocyte cells; and

c) transplanting said [transfected connective tissue] transfected/transduced chondrocyte cells without scaffolding by intraarticular injection to an [arthritic] osteoarthritic joint space of a mammalian host, such that expression of said DNA sequence within said joint space results in regenerating connective tissue.

2. (Amended) The method of claim [1] 13, wherein said recombinant viral vector is a retroviral vector.

3. (Amended) The method of claim [1] 13, wherein said recombinant vector is a plasmid vector.

4. (Amended) The method of claim [1] 13, wherein said population of [transfected connective tissue] transfected/transduced chondrocyte cells are stored prior to transplantation.

5. (Amended) The method of claim 4, wherein said population of [transfected connective tissue] transfected/transduced chondrocyte cells are stored in 10% DMSO under liquid nitrogen prior to transplantation.

13. (Amended) A method of regenerating hyaline cartilage, comprising:

a) generating a recombinant viral or plasmid vector comprising a DNA sequence encoding [a member of a] transforming growth factor [superfamily of proteins] β1 (TGF-β1) or BMP operatively linked to a promoter;

b) [transfecting] transfecting/transducing *in vitro* a population of [cultured connective tissue] chondrocyte cells with said recombinant vector, resulting in a population of [transfected] transfected/transduced connective tissue cells; and

c) [transplanting said transfected connective tissue cells by intraarticular injection to joint space of a mammalian host, such that expression of said DNA sequence within said joint space results in regenerating hyaline cartilage] injecting a composition comprising the transfected/transduced population of chondrocyte cells and a pharmaceutically acceptable carrier without scaffolding into a joint space of a mammal such that expression of the DNA sequence encoding TGFβ1 or BMP within the joint space occurs resulting in the generation of hyaline cartilage in the joint space.

14. (Amended) The method of claim [1] 13, wherein said transfection is accomplished by liposome encapsulation, calcium phosphate coprecipitation, electroporation and DEAE-dextran mediation.